# Appendix C

**Summary Review Document**

**Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Using *In Vitro* Alternative Methods**

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# Summary Review Document
# Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Using *In Vitro* Alternative Test Methods

**Interagency Coordinating Committee on the Validation of Alternative Methods**

**National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods**

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**National Institutes of Health**
**U.S. Public Health Service**
**Department of Health and Human Services**

**2010**

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# List of Abbreviations and Acronyms

| | |
|---|---|
| %CV | Percent coefficient of variation |
| AMCP | Antimicrobial cleaning product |
| ATWG | Alternative Testing Working Group |
| BCOP | Bovine corneal opacity and permeability |
| BRD | Background review document |
| CFR | Code of Federal Regulations |
| CM | Cytosensor® Microphysiometer |
| COLIPA | European Cosmetic, Toiletry and Perfumery Association |
| CPSC | U.S. Consumer Product Safety Commission |
| CTFA | Cosmetic, Toiletry and Fragrance Association |
| CV | Coefficient of variation |
| EC/HO | European Commission/British Home Office |
| ECVAM | European Centre for the Validation of Alternative Methods |
| EO | EpiOcular™ |
| EPA | U.S. Environmental Protection Agency |
| ESAC | European Centre for the Validation of Alternative Methods Scientific Advisory Committee |
| $ET_{50}$ | Time needed to reduce cell viability by 50% |
| FDA | U.S. Food and Drug Administration |
| FHSA | Federal Hazardous Substances Act |
| FIFRA | Federal Insecticide, Fungicide and Rodenticide Act |
| FR | *Federal Register* |
| GHS | United Nations Globally Harmonized System of Classification and Labelling of Chemicals |
| GLP | Good Laboratory Practice |
| ICCVAM | Interagency Coordinating Committee on the Validation of Alternative Methods |
| ILS | Integrated Laboratory Systems, Inc. |
| ISO | International Organization for Standardization |
| IVIS | *In vitro* irritancy score |
| JaCVAM | Japanese Center for the Validation of Alternative Methods |
| LVET | Low volume eye test |
| MAS | Maximum average score |
| $MRD_{50}$ | Estimated concentration of a test substance needed to reduce the basal metabolic rate of L929 cells by 50% |
| NICEATM | National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods |
| NIEHS | National Institute of Environmental Health Sciences |
| NTP | National Toxicology Program |

| | |
|---|---|
| OECD | Organisation for Economic Co-operation and Development |
| OPP | Office of Pesticide Programs |
| OPPTS | Office of Prevention, Pesticides and Toxic Substances |
| OSHA | Occupational Safety and Health Administration |
| OTWG | Ocular Toxicity Working Group |
| PPDC | Pesticide Product Dialog Committee |
| REACH | Registration, Evaluation, and Authorisation of Chemicals |
| SACATM | Scientific Advisory Committee on Alternative Toxicological Methods |
| SD | Standard deviation |
| SM | Silicon Microphysiometer |
| SRD | Summary review document |
| TG | Test guideline |
| TNO | TNO Nutrition and Food Research Institute (Netherlands) |
| TSCA | Toxic Substances Control Act |
| U.K. | United Kingdom |
| U.N. | United Nations |
| U.S. | United States |
| U.S.C. | United States Code |
| w/v | Weight-to-volume ratio |

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# Preface

Commercial and household cleaning products require labeling to indicate if they are hazardous to the consumer and have the potential to cause injury during handling or use, including possible ingestion by children. The U.S. Consumer Product Safety Commission (CPSC) typically regulates these cleaning products. However, inclusion of an antimicrobial claim in such cleaning products necessitates their registration as antimicrobial pesticides with the U.S. Environmental Protection Agency (EPA). Accordingly, to comply with EPA classification and labeling requirements for eye irritation (EPA 2003a), a product manufacturer must test these cleaning products in the Draize rabbit eye test (Draize et al. 1944) to adequately characterize their ocular hazard potential.

In June 2004, the EPA contacted the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), which administers the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and provides scientific support for ICCVAM activities, to seek the assistance in a technical assessment of an *in vitro* testing strategy that would meet their need to evaluate, categorize, and label antimicrobial cleaning products (AMCPs) for eye irritation. Subsequently, the Alternative Testing Working Group (ATWG), a consortium of seven consumer product companies (Clorox, Colgate-Palmolive, Dial, EcoLabs, JohnsonDiversey, Procter & Gamble, and SC Johnson), developed a testing strategy that is comprised of three *in vitro* test methods (i.e., bovine corneal opacity and permeability [BCOP], Cytosensor® Microphysiometer [CM], and EpiOcular™ [EO]) for this limited group of products. The Institute for In Vitro Sciences, Inc., which coordinated the ATWG collaboration, performed additional testing to complete parallel sets of *in vivo* and *in vitro* data and described the testing strategy in a background review document (BRD). The EPA and the ATWG requested that NICEATM and ICCVAM use the information in the AMCP BRD to conduct a technical review of the scientific validity of the AMCP testing strategy. The EPA and the ATWG sought to determine whether EPA could be assured with a reasonable degree of certainty that the AMCP testing strategy would be useful for making hazard classification and labeling decisions for AMCPs in order to appropriately inform users. A *Federal Register* (FR*)* notice (70 FR 13512) issued on March 21, 2005, requested relevant data and nominations for potential peer review panel members.

NICEATM received an initial draft of the AMCP BRD from the Institute for In Vitro Sciences, Inc., on December 27, 2007; formal transmittal letters were received from the Institute for In Vitro Sciences, Inc., and the EPA on January 8 and 10, 2008, respectively. On March 17, 2008, following a preliminary review of the AMCP BRD, the ICCVAM Ocular Toxicity Working Group (OTWG) requested additional information and data from the Institute for In Vitro Sciences, Inc. The additional data, which were necessary to complete an evaluation, were received on April 4, 2008.

On April 4, 2008, *Federal Register* notice (73 FR 18535) requested relevant data and nominations for potential peer review panel members. On June 23–24, 2008, the OTWG and ICCVAM assigned this activity a high priority following consideration of comments from the public and ICCVAM's advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). The Institute for In Vitro Sciences, Inc. submitted a final revised AMCP BRD on July 21, 2008. A supplement to the AMCP BRD, which included reliability analyses for the *in vitro* test methods (i.e., BCOP, CM, and EO), was submitted on October 8, 2008.

The OTWG worked with NICEATM to prepare this summary review document (SRD), which summarizes the current validation status of the AMCP testing strategy based on information in the AMCP BRD and other related information and data obtained by NICEATM. This AMCP SRD also provides similar information for an alternate AMCP testing strategy. This AMCP SRD summarizes the information from the AMCP BRD needed to evaluate the validation status of each of the *in vitro* test methods, the AMCP testing strategy, and the alternate AMCP testing strategy and forms the basis for the ICCVAM test method recommendations.

An independent international scientific peer review panel met in public forum on May 19–21, 2009, to develop conclusions and recommendations for the AMCP testing strategy. The Panel included expert scientists nominated by the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM). The Panel considered this AMCP SRD and evaluated the extent to which the available information supported the draft ICCVAM test method recommendations. ICCVAM considered the conclusions and recommendations of the Panel, along with comments received from the public and the SACATM, before finalizing this AMCP SRD and test method recommendations.

We gratefully acknowledge the organizations and scientists who provided data and information for this document. We also acknowledge the efforts of those individuals who helped prepare this AMCP SRD. These include Dr. Jill Merrill (U.S. Food and Drug Administration Center for Drug Evaluation and Research) and Dr. Karen Hamernik (EPA, to April 2009) for serving as Co-chairs of the OTWG and ICCVAM representatives who reviewed and provided comments throughout the evaluation process. We also acknowledge the following staff from the NICEATM support contractor, Integrated Laboratory Systems, Inc.: Dr. David Allen, Dr. Jonathan Hamm, Nelson Johnson, Dr. Brett Jones, Dr. Elizabeth Lipscomb, and James Truax. Finally, we thank ECVAM liaisons Dr. João Barroso, Dr. Thomas Cole, and Dr. Valerie Zuang and JaCVAM liaison Dr. Hajime Kojima for their participation.

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# Executive Summary

The Alternative Testing Working Group, a consortium of consumer product companies, developed a testing approach for antimicrobial cleaning products (AMCPs). In 2007, the Institute for In Vitro Sciences, Inc. (IIVS), described the approach in a background review document (BRD). The AMCP testing strategy consists of three *in vitro* test methods: bovine corneal opacity and permeability (BCOP), Cytosensor® Microphysiometer (CM), and EpiOcular™ (EO). The AMCP BRD includes a detailed protocol for each test method. Decision criteria were developed for each test method to correspond to the four ocular hazard categories in the U.S. Environmental Protection Agency (EPA) classification system (EPA Category I, II, III, and IV [EPA 2003a]). These test methods use a variety of endpoints to predict the potential of test substances to cause eye irritation.

### *The AMCP Testing Strategy: Combining the BCOP, CM, and EO Test Methods*

The BCOP includes two primary endpoints, opacity and permeability. Opacity and permeability measurements are used to calculate an *in vitro* irritancy score (IVIS).[1] Histopathology evaluation of the affected tissue is an optional endpoint. Substances with an IVIS ≥75 are classified as EPA Category I; those with an IVIS ≥25 and <75 are EPA Category II; and substances with an IVIS <25 are EPA Category III. If a test substance produces an IVIS <75, further assessment using histopathology evaluation can determine whether it meets the criteria for classification as EPA Category I, II, or III. Because the data points from EPA Category III and Category IV overlap and it's impossible to assign a cutoff value, the AMCP BRD does not propose BCOP decision criteria for EPA Category IV.

The endpoint for the CM test method is the estimated concentration of a test substance needed to reduce the basal metabolic rate of L929 cells by 50% (the $MRD_{50}$). Substances with an $MRD_{50}$ value <2 mg/mL are classified as EPA Category I; those with an $MRD_{50}$ ≥2 mg/mL and <80 mg/mL are EPA Category III; and substances with an $MRD_{50}$ ≥80 mg/mL are classified as EPA Category IV. The AMCP BRD does not propose CM decision criteria for EPA Category II because the data points from EPA Category I and Category II overlap making it impossible to assign a cutoff value.

The endpoint for the EO test method is the time needed to reduce cell viability by 50% ($ET_{50}$). Substances with an $ET_{50}$ <4 minutes are classified as EPA Category I; those with an $ET_{50}$ ≥4 minutes and <70 minutes are EPA Category III; and substances with an $ET_{50}$ ≥70 minutes are classified as EPA Category IV. The AMCP BRD does not propose decision criteria for the EO test method for EPA Category II because the database includes only one EPA Category II substance.

The AMCP BRD proposes starting with different test methods depending on the chemical properties of the test substance. If the test substance is an oxidizer, which suggests that it will be an ocular corrosive or severe irritant, it is first tested in the BCOP test method. As noted above, test substances that produce an IVIS ≥75 would be classified as EPA Category I. If a test substance produces an IVIS <75, further assessment using histopathology evaluation can determine whether it meets the criteria for classification as EPA Category I, II, or III.

To determine whether the test substance is EPA Category III or IV, the test substance is subsequently tested in either the CM or EO test method to determine the final hazard category. The choice of test method depends on the chemical properties of the test substance. If the test substance is water soluble, it can be tested in either the CM test method or the EO test method. If it is water insoluble, it must be tested in the EO test method to determine the final hazard classification.

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[1]The *in vitro* irritancy score (IVIS) is the sum of the mean corrected opacity value (± standard deviation [SD]) and 15 times the mean corrected permeability value ($OD_{490}$ units ± SD).

### *Alternate AMCP Testing Strategy: Combining the BCOP and EO Test Methods*

None of the 228 substances in the validation database has been tested in all three of the *in vitro* test methods included in the AMCP testing strategy. ICCVAM also had concerns about the validation status of the low volume eye test (LVET),[2] which was used as the *in vivo* reference test method for all of the CM test method data. Therefore, the Interagency Coordination Committee on the Validation of Alternative Methods (ICCVAM) evaluated an alternate AMCP testing strategy that included only the BCOP and the EO test methods. In this alternate AMCP testing strategy, the BCOP test method would be used to identify EPA Category I and II substances and the EO test method would be used to identify EPA Category III and IV substances.

Testing in the alternate AMCP testing strategy could proceed by one of two approaches: (1) test in the BCOP test method first and then in the EO test method or (2) test in the EO test method first and then in the BCOP test method. Using the first approach, the BCOP test method would classify all EPA Category I and II substances. All other substances would then be tested in the EO test method and classified as either EPA Category III or IV. Using the second approach, substances would first be tested in the EO test method, which would classify all EPA Category III and IV substances. All other substances would then be tested in the BCOP test method and classified as either EPA Category I or II.

## Validation Database

A total of 228 substances were included in the validation database for the AMCP BRD. These include 68 substances tested in the BCOP test method, 105 substances tested in the CM test method, and 55 substances tested in the EO test method. None of the 228 substances has been tested in all three *in vitro* test methods. According to the submitter, "a minimum 28 of the materials are EPA registered AMCPs, with eight additional materials being in-use dilutions of concentrates which are EPA registered" (Rodger Curren, IIVS, Inc., personal communication).

The distribution of product categories differed among the test methods. Most of the 105 substances tested in the CM test method are surfactants (78%). The substances tested in the BCOP and EO test methods are relatively equally distributed among alkalis, oxidizers, solvents, and surfactants (approximately 20% to 30% each).

Only 28 AMCPs have been tested in both the BCOP and EO test methods.

## *In Vivo* Reference Data

The test method protocol used to generate the *in vivo* reference data varied among the 228 substances. Among the 68 substances tested in the BCOP test method, 85% were also tested in the traditional Draize rabbit eye test protocol (i.e., OECD TG 405 [OECD 2002]). Another 12% were tested with a nontraditional protocol (i.e., application volume of 30 μL instead of 100 μL or application as an aerosol spray). The remaining 3% were tested in the LVET.

Among the 55 substances tested in the EO test method, 55% were tested in the Draize rabbit eye test, and 45% were tested in the LVET. All 105 of the substances tested in the CM test method were tested in the LVET.

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[2] The LVET is a modification to the rabbit eye test that involves application of 10 μL of the test substance directly to the corneal surface instead of 100 μL of the test substance applied into the conjunctival sac.

## Test Method Accuracy

### *The Bovine Corneal Opacity and Permeability Test Method*

The validation database of 66 substances tested in both the BCOP test method and the Draize rabbit eye test showed 55% accuracy (36 of 66 tests agreed in overall EPA classification) (**Table 1**). The BCOP test method correctly classified only 60% as EPA Category II and 50% as EPA Category III. However, the BCOP test method correctly identified 90% of the EPA Category I substances. Because the AMCP BRD does not propose BCOP decision criteria for EPA Category IV, all 19 substances were overpredicted.

### *The Cytosensor Microphysiometer Test Method*

The validation database includes 105 unique substances tested in both the CM test method and the LVET (**Table 1**). Three substances were tested twice for a total of 108 tests. These tests had 30% accuracy (32 of 108 tests agreed in overall classification of EPA Category I, II, III, or IV). The CM test method overclassified the majority of EPA Category II, III, and IV substances in the database: 100% of the EPA Category II substances, 67% of the EPA Category III substances, and 89% of the EPA Category IV substances. Because the AMCP BRD does not propose CM test method decision criteria for EPA Category II, the CM test method overclassified all EPA Category II and III substances as EPA Category I.

### *The EpiOcular Test Method*

Among the 55 substances tested in the EO test method (**Table 1**), 30 were also tested in the Draize rabbit eye test (29 qualified for EPA hazard classification) and 25 were tested in the LVET. Those tested in both the EO test method and the Draize rabbit eye test had 76% accuracy (22 of 29 tests agreed in overall classification of EPA Category I, II, III, or IV). The EO test method correctly identified three (75%) of the four substances categorized as EPA Category III by the Draize rabbit eye test. The EO test method correctly identified 44% of the nine EPA Category IV substances. Four of the five substances incorrectly identified by the EO test method were overclassified as EPA Category III. The EO test method overclassified the remaining substance as EPA Category I. All of the EPA Category I substances were correctly identified.

Among the 25 substances tested in both the EO test method and the LVET (**Table 1**), the EO test method correctly classified 44%. The EO test method correctly identified 67% of the 12 substances classified as EPA Category III by the LVET. None of the nine EPA Category IV substances was correctly identified; 44% were overclassified as EPA Category III; and 56% were overclassified as EPA Category I. The EO test method correctly identified all three of the substances classified as EPA Category I by the LVET.

### *AMCP Testing Strategy: Combining the BCOP, CM, and EO Test Methods*

As explained above, none of the 228 substances included in the AMCP BRD was tested in all three of the *in vitro* test methods proposed for the AMCP testing strategy. Therefore, no data are available to characterize the actual performance of a testing strategy that includes the BCOP, CM, and EO test methods.

### *Alternate AMCP Testing Strategy: Combining the BCOP and EO Test Methods*

The BCOP and EO test methods were both used to test 28 substances for which Draize rabbit eye test data were available. This suggested an alternate AMCP testing strategy in which the BCOP test method might be used to identify EPA Category I or Category II substances and the EO test method might be used to identify EPA Category III or Category IV substances. ICCVAM evaluated the data based on two approaches: (1) test in the BCOP test method first and then in the EO test method or (2) test in the EO test method first and then in the BCOP test method.

**Table 1 Performance of AMCPs in the Bovine Corneal Opacity and Permeability, Cytosensor Microphysiometer, and EpiOcular Test Methods Compared to the Draize Rabbit Eye Test or the Low Volume Eye Test as Reported in the AMCP BRD Using the EPA Classification System**

| *In Vitro* Test Method | *In Vivo* Test Method | Overall Classification | Performance of the *In Vitro* Test Method Compared to the *In Vivo* Reference Test Method Using the EPA Classification System | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | I | | II | | | III | | | IV | |
| | | | Actual | Under | Over | Actual | Under | Over | Actual | Under | Over | Actual |
| BCOP[1] | Draize | 55% (36/66) | 90% (27/30) | 10% (3/30) | 20% (1/5) | 60% (3/5) | 20% (1/5) | 50% (6/12) | 50% (6/12) | 0% (0/12) | 100% (19/19) | 0% (0/19) |
| CM[2] | LVET | 30% (32/108) | 100% (9/9) | 0% (0/9) | 100% (11/11) | 0% (0/11) | 0% (0/11) | 67% (40/60) | 33% (20/60) | 0% (0/60) | 89% (25/28) | 11% (3/28) |
| EO[3] | Draize | 76% (22/29) | 100% (15/15) | 0% (0/15) | 0% (0/1) | 0% (0/1) | 100% (1/1) | 25% (1/4) | 75% (3/4) | 0% (0/4) | 56% (5/9) | 44% (4/9) |
| EO[4] | LVET | 44% (11/25 | 100% (3/3) | 0% (0/3) | 100% (1/1) | 0% (0/1) | 0% (0/1) | 33% (4/12) | 67% (8/12) | 0% (0/12) | 100% (9/9) | 0% (0/9) |

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; CM = Cytosensor Microphysiometer; EO = EpiOcular; EPA = U.S. Environmental Protection Agency; $ET_{50}$ = estimated time to decrease keratinocyte viability in the EO test method by 50%; IVIS = *in vitro* irritancy score; LVET = low volume eye test; $MRD_{50}$ = concentration of test substance that decreases the metabolic rate by 50% determined by a plot of the concentration-response curve.

[1] Classification of the BCOP data was based on IVIS ≥75 = EPA Category I; IVIS ≥25 and <75 = EPA Category II; IVIS <25 = EPA Category III. The BCOP test method was not proposed to identify EPA Category IV. All BCOP classifications, including high-solvent substances, used a 10-minute exposure time. The database comprised 66 substances tested in both the BCOP test method and the Draize rabbit eye test.

[2] Classification of the CM data was based on $MRD_{50}$ <2 mg/mL = EPA Category I; $MRD_{50}$ ≥2mg/mL and <80 mg/mL = EPA Category III; $MRD_{50}$ ≥80 mg/mL = EPA Category IV. The CM test method was not proposed to identify EPA Category II. The database consisted of 108 substances tested in both the CM test method and in the LVET (105 different substances because three substances were tested twice).

[3] Classification of the EO data was based on $ET_{50}$ <4 min = EPA Category I; $ET_{50}$ ≥4 min and <70 min = EPA Category III; $ET_{50}$ ≥70 min = EPA Category IV. The EO test method was not proposed to identify EPA Category II. The database consisted of 29 substances tested in both the EO test method and the Draize rabbit eye test that qualified for EPA hazard classification (i.e., one substance producing a Draize score greater than 1 was not evaluated through day 21 as required by EPA).

[4] Classification of the EO data was based on $ET_{50}$ <4 min = EPA Category I; $ET_{50}$ ≥4 min and <70 min = EPA Category III; $ET_{50}$ ≥70 min = EPA Category IV. The EO test method was not proposed to identify Category II. The database consisted of 25 substances tested in both the EO test method and the LVET.

For the first approach, ICCVAM evaluated the BCOP test method's ability to identify substances as either EPA Category I or Category II. All 15 substances that were classified as EPA Category I or II in the BCOP test method were removed from the database. The remaining 13 substances were then evaluated in the EO test method for identifying EPA Category III or IV substances. The reverse was done for the second approach: the EO test method was evaluated for its ability to classify substances as either EPA Category III or IV. All 13 substances that had been classified as EPA Category III or IV by the EO test method were removed from the database. The remaining 15 substances were then evaluated in the BCOP test method for identifying EPA Category I or II substances.

The alternate AMCP testing strategy performed the same regardless of which approach was used (**Table 2**). The alternate AMCP testing strategy correctly classified 79% of the substances, which included all 14 of the EPA Category I substances, all four of the EPA Category III substances, and four of the nine (44%) EPA Category IV substances. The one EPA Category II substance was underpredicted as EPA Category III.

## Test Method Reliability

### *The Bovine Corneal Opacity and Permeability Test Method*

In the AMCP BRD, intralaboratory repeatability for the BCOP test method (i.e., comparison of within-experiment runs of a test substance) was determined for 67 AMCPs (four substances have repeat tests) as the mean percent coefficient of variation (%CV) for opacity, permeability, and IVIS. Because scores in the very low range significantly affect %CVs, the mean %CVs for materials with an IVIS $\leq 10$ (arbitrarily set in the AMCP BRD) were excluded from the overall mean %CV calculations. The overall mean %CVs for opacity, permeability, and IVIS were 21%, 25%, and 18%, respectively.

These 67 test substances, tested in a total of 75 runs, were also evaluated for their agreement in the EPA (EPA 2003a) and Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) ocular hazard classification systems. The EPA and GHS classification systems had 100% agreement in 84% (63 of 75) of test runs, 67% agreement in 15% (11 of 75) of test runs, and 60% agreement in 1% (1 of 75) of test runs. Among the 12 test runs that did not have 100% agreement, seven substances had reactive chemistries, two were alkalis, two were surfactants, and one was an acid.

Intralaboratory repeatability for the BCOP test method was determined for non-AMCPs classified as severe or ocular corrosives in three BCOP studies, which tested from 16 to 52 substances (ICCVAM 2006a). The mean %CVs for IVIS ranged from 39% to 71%.

Intralaboratory reproducibility for the BCOP test method (i.e., comparison of between-experiment runs of a test substance) was determined for five AMCPs as the mean %CV for IVIS. In two to six experiments, the mean %CV for IVIS was 20%. The agreement in the EPA (EPA 2003a) and GHS (UN 2007) ocular hazard classification systems for these five test substances was 100%.

Intralaboratory reproducibility for the BCOP test method was also determined for non-AMCPs classified as severe ocular irritants or ocular corrosives by the BCOP test method (ICCVAM 2006a). One of the two studies consisted of 25 surfactant-based personal-care cleaning formulations. The mean %CV for permeability values in that study was 33%. In the second study of 16 substances, the mean %CV for IVIS ranged from 13% to 15%.

Interlaboratory reproducibility for the BCOP test method (i.e., comparison of runs of a test substance between different laboratories) cannot be specifically determined for AMCPs in the BRD because only one laboratory conducted the testing.

**Table 2 AMCPs Tested in Both the BCOP and EO Test Methods: Performance Using the Alternate AMCP Testing Strategy**

| EPA | Overall Classification | Draize | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | II | | | III | | | IV | |
| | | Actual | Under | Over | Actual | Under | Over | Actual | Under | Over | Actual |
| Approach 1 | 79% (22/28) | 100% (14/14) | 0% (0/14) | 0% (0/1) | 0% (0/1) | 100% (1/1) | 0% (0/4) | 100% (4/4) | 0% (0/4) | 56% (5/9) | 44% (4/9) |

| EPA | Overall Classification | Draize | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | II | | III | | | | IV | |
| | | Actual | Under | Over | Actual | Under | Over | Actual | Under | Over | Actual |
| Approach 2 | 79% (22/28) | 100% (14/14) | 0% (0/14) | 0% (0/1) | 0% (0/1) | 100% (1/1) | 0% (0/4) | 100% (4/4) | 0% (0/4) | 56% (5/9) | 44% (4/9) |

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; EO = EpiOcular; EPA = U.S. Environmental Protection Agency.

Approach 1 = test in the BCOP test method first to identify EPA Category I or II, and then in the EO test method to identify EPA Category III or IV.

Approach 2 = test in the EO test method first to identify EPA Category III or IV, and then in the BCOP test method to identify EPA Category I or II.

Three studies (3–12 laboratories each) were used to determine interlaboratory reproducibility in non-AMCPs classified as severe or ocular corrosives by the BCOP test method (ICCVAM 2006a). The mean %CV for IVIS ranged from 25% to 36%. These test substances were also evaluated (ICCVAM 2006a) for their agreement with the EPA (EPA 2003a), GHS (UN 2007), and European Union (EU 2001) ocular hazard classification systems.

***The Cytosensor Microphysiometer Test Method***

Reliability for the CM test method could not be evaluated specifically for AMCPs due to insufficient data. However, the reliability of the CM test method was evaluated in non-AMCPs.

Intralaboratory repeatability for the CM test method was evaluated for non-AMCPs in seven studies of 1 to 35 test substances each. The mean %CV for $MRD_{50}$ values for all materials tested, including surfactant and nonsurfactant materials, ranged from 6% to 25%.

The intralaboratory reproducibility of the CM test method for non-AMCPs in one laboratory (16 substances). The mean %CV for $MRD_{50}$ values for all materials tested, including surfactant and nonsurfactant materials, was 25%.

Interlaboratory reproducibility for this test method was determined for non-AMCPs in two studies at two to four laboratories each. The mean %CV for $MRD_{50}$ values for all materials tested, including surfactant and nonsurfactant materials, ranged from 17% to 51%, with nonsurfactant materials having a higher mean %CV in each study.

***The EpiOcular Test Method***

Intralaboratory repeatability for the EO test method was determined specifically for a subset of 15 AMCPs presented in the AMCP BRD. The mean %CV for $ET_{50}$ values ranged from 0% to 62%.

The extent of agreement between the EPA and GHS ocular hazard classification systems (EPA 2003a; UN 2007) was evaluated for three AMCPs that were tested more than once by IIVS. All three AMCPs had 100% agreement for both hazard classification systems.

Intralaboratory reproducibility for the EO test method was also determined from repeat testing of a single substance, 0.3% Triton X-100. Data were presented as combined data from MatTek Corporation and IIVS (9-year period) and from IIVS only (8-year period). The mean %CVs for $ET_{50}$ values were 21% and 22%, respectively.

Interlaboratory reproducibility for the EO test method cannot be determined specifically for the AMCPs presented in the AMCP BRD because only one laboratory conducted the testing. However, interlaboratory reproducibility for this test method has been determined for non-AMCPs in a multiphase validation study of surfactants and surfactant-containing products (73 substances). The study is summarized in the AMCP BRD. Mean %CVs ranged from 12% to 18%. It should be noted, however, that this reproducibility evaluation did not use a calculated $ET_{50}$ value to predict the ocular hazard classification (i.e., EPA Category I, II, III, and IV), as specified in the protocol included in the AMCP BRD. Instead, it is based on an EO protocol that uses relative percent viability to classify irritancy (i.e., irritant vs. nonirritant).

These same non-AMCP test substances were also evaluated for agreement with the EPA and GHS ocular hazard classification systems (EPA 2003a; UN 2007). This analysis is summarized in a supplement to the AMCP BRD. Using the EPA and GHS classification systems in Phase II of the validation study, four laboratories produced 100% agreement for 74% of the 19 substances, 75% agreement for 11% of the substances, and 50% agreement for 16% of the substances. In Phase III at two laboratories, 94% of the 54 substances had 100% agreement, and the remaining 6% (3 substances) had 0% agreement.

**Animal Welfare Considerations**

Both of the AMCP testing strategies are non-animal approaches for the classification and labeling of AMCPs. Bovine eyes used in the BCOP test method are obtained post mortem from animals being used for food. The CM test method uses a mouse cell line that can be purchased. The EO test method uses primary human keratinocytes obtained from human donors during routine surgical procedures.

**Practical Considerations**

The BCOP test method can be completed in one day, but histopathology evaluation may require an additional four weeks.

The CM test method, including multiple runs of the test material, can be completed in a single workday. However, the instrument for the CM test method has been discontinued.

The EO test method uses tissue that is commercially available from MatTek Corporation (Ashland, MA). The cost of the EO test method is similar to or less than that of a Draize rabbit eye test. Although it may take several weeks to procure tissue from the MatTek Corporation, the EO test method may be run in less time than the Draize rabbit eye test or the LVET.

# 1.0 Introduction and Rationale for the Use of a Testing Strategy for U.S. Environmental Protection Agency Classification and Labeling of Antimicrobial Cleaning Products

## 1.1 Historical Background of *In Vitro* Ocular Corrosion and Irritation Test Methods and the Rationale for Their Development

Over the years, legislative statutes have been enacted that enable government agencies to regulate a variety of substances that pose a potential risk to ocular health. **Table 1-1** provides a synopsis of current U.S. regulatory laws that pertain to ocular corrosion and irritation.

**Table 1-1 Summary of Current U.S. Legislation Related to Ocular Health[1]**

| Legislation (Year of Initial Enactment) | Agency | Substance |
|---|---|---|
| Food, Drug and Cosmetic Act (1938) | FDA | Pharmaceuticals and cosmetics |
| FIFRA (1947) and Federal Environmental Pesticide Control Act (1972) | EPA | Pesticides |
| FHSA (1964) | CPSC | Household products |
| FHSA (1964) and TSCA (1976) | Department of Agriculture and EPA | Agricultural and industrial chemicals |
| Occupational Safety and Health Act (1970) | OSHA | Occupational materials |
| Clean Air Act Amendments (1990) | Chemical Safety and Hazard Investigation Board and EPA | Accidentally released chemicals and air pollutants |

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration, FHSA = Federal Hazardous Substances Act; FIFRA = Federal Insecticide, Fungicide, and Rodenticide Act; OSHA = Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act.

[1] Adapted from Wilhelmus (2001).

Exposing rabbit eyes to a test substance is the primary method for assessing the ocular hazard potential of substances that may come near or in contact with the eye of a human. The test method currently accepted by U.S. Federal and international regulatory agencies (CPSC 1995; EPA 1998; OECD 2002) is the Draize rabbit eye test (Draize et. al. 1944). In the Draize rabbit eye test, a test substance is applied to the lower conjunctival sac of one eye of a rabbit and compared to the contralateral eye, which serves as a negative control. The eyes of each rabbit are examined for adverse corneal (i.e., opacity and area of involvement), iridal, or conjunctival (i.e., redness, chemosis, and discharge) effects for a period up to 21 days after exposure to the test substance.

The Draize rabbit eye test can identify both irreversible (corrosive) and reversible ocular effects. The wide ranges used for scoring a majority of these lesions permit categorization of the severity of reversible effects as moderate, mild, or nonirritant (see U.S. Environmental Protection Agency [EPA] Ocular Classification System discussed below). Current EPA ocular testing guidelines and the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) indicate that if serious ocular damage is anticipated (e.g., irreversible adverse effects on day 21), then a test on a single animal may be considered. If serious damage is observed, then no further

animal testing is necessary (EPA 1998; UN 2007). If no serious damage is observed, additional test animals (1 or 2 rabbits) may be evaluated sequentially until concordant responses are observed (UN 2007).

The ocular classification systems vary depending on the regulatory agency's legislative mandate and goals for protecting human health (**Table 1-2)**. The EPA classification system and testing guidelines (EPA 1998, 2003a) are based on the most severe response in one animal in a group of three or more animals. This classification system considers the kinds of ocular effects produced, as well as the reversibility and the severity of the effects. The EPA classifies substances into four ocular irritant categories (i.e., EPA Category I, II, III, and IV) (**Table 1-2**) (EPA 2003a). The EPA defines Category I substances as corrosive or severe irritants, while classification in EPA Category II, III, or IV is based on decreasing severity of ocular lesions, as well as the time required for the ocular lesions to clear. Irritation that clears in 8 to 21 days is classified as EPA Category II, while irritation that clears within 7 days is classified as EPA Category III. For EPA Category IV substances, irritation clears within 24 hours.

To harmonize the classification of ocular irritants internationally, the GHS classification system (UN 2007) includes two categories (**Table 1-2**), one for irreversible effects on the eye/serious damage to the eye (GHS Category 1) and one for reversible effects on the eye (GHS Category 2). Classification is based on the severity of the lesions and/or the duration of their persistence. Reversible effects are further classified based on the duration as GHS Category 2A ("irritating to eyes" referring to an effect that reverses within 21 days) and GHS Category 2B ("mildly irritating to eyes" referring to an effect that reverses within 7 days).

The U.S. Federal Hazardous Substances Act (FHSA; FHSA 1964) (CPSC 1995) and the European Union (EU; EU 2001) also have classification criteria for ocular irritation. However, because this evaluation focuses on ocular hazard classification according to the EPA and GHS systems, the criteria for the FHSA and EU systems will not be discussed. Additional details on these systems can be found in the BCOP BRD (ICCVAM 2006a).

Recently, the EPA requested that the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluate a non-animal strategy to classify and label antimicrobial cleaning products (AMCPs). This testing strategy was developed by the Alternative Testing Working Group (ATWG), composed of seven consumer product companies (Clorox, Colgate-Palmolive, Dial, EcoLabs, JohnsonDiversey, Procter & Gamble, and SC Johnson). The AMCP testing strategy includes three *in vitro* test methods (bovine corneal opacity and permeability [BCOP], Cytosensor Microphysiometer [CM)], and EpiOcular [EO]). *In vitro* data were paired with *in vivo* data obtained in either the Draize rabbit eye test or the low volume eye test (LVET).

On behalf of the ATWG, the Institute for In Vitro Sciences, Inc. submitted an AMCP background review document (BRD) (**Annex I**) and AMCP BRD Supplement (**Annex II**), which provided additional information on the reliability for each *in vitro* test method, to ICCVAM for review of the validation status of the AMCP testing strategy. The EPA and the ATWG requested that NICEATM and ICCVAM use information within the AMCP BRD to conduct a technical review of the AMCP testing strategy to determine whether ICCVAM could assure the EPA with a reasonable degree of certainty that the AMCP testing strategy would help the EPA determine AMCP labeling that would appropriately inform users.

This AMCP summary review document (SRD) summarizes the available data and information regarding the usefulness and limitations of the AMCP testing strategy as described in the AMCP BRD and an alternate AMCP testing strategy that uses only the BCOP and EO test methods.

**Table 1-2 Ocular Toxicity Classification Systems**

| Regulatory Agency (Authorizing Act) | Number of Animals | Observation Days (after treatment) | Mean Score Taken? | Positive Response | Classification Criteria |
|---|---|---|---|---|---|
| EPA (FIFRA, Federal Environmental Pesticide Control Act, and TSCA) | At least 3 | 1 hr, 1, 2, 3, 7, and 21 | No | Maximum score in an animal used for classification<br><br>Opacity or Iritis ≥1 or Redness or Chemosis ≥2 | One or more positive animals needed for classification in categories below.<br>Category:<br>I = Corrosive, corneal involvement, or irritation persisting more than 21 days<br>II = Corneal involvement or irritation clearing in 8–21 days<br>III = Corneal involvement or irritation clearing in 7 days or less<br>IV = Minimal effects clearing in less than 24 hours<br>**Definition of Full Reversal:**<br>Opacity and Iritis scores = 0 and Redness and Chemosis scores ≤1 |
| GHS: Irreversible Eye Effects | 3 | 1, 2, 3 (observation until day 21) | Yes | Mean animal values (over days 1, 2, and 3) of:<br>Opacity ≥3 and/or Iritis ≥1.5 | At least 2 positive response animals = Eye Irritant Category 1<br>At least 1 animal with Opacity, Chemosis, Redness, or Iritis scores >0 on day 21 = Eye Irritant Category 1<br>**Definition of Full Reversal:**<br>Opacity, Iritis, Redness, and Chemosis scores = 0 |
| GHS: Reversible Eye Effects | 3 | 1, 2, 3 (observation until day 21) | Yes | Mean animal values (over days 1, 2, and 3) of:<br>Opacity or Iritis ≥1 or<br>Redness or Chemosis ≥2<br>and the effect fully reverses in 7 or 21 days | At least 2 positive response animals and the effect fully reverses in 21 days = Eye Irritant Category 2A<br>At least 2 positive response animals and effect fully reverses in 7 days = Eye Irritant Category 2B<br>**Definition of Full Reversal:**<br>Opacity, Iritis, Redness, and Chemosis scores = 0 |

Abbreviations: EPA = U.S. Environmental Protection Agency; FIFRA = Federal Insecticide, Fungicide, and Rodenticide Act; GHS = Globally Harmonised System of Classification and Labelling of Chemicals; TSCA = Toxic Substances Control Act.

## 1.2 Regulatory Rationale and Applicability

The U.S. Consumer Product Safety Commission (CPSC) typically regulates commercial and household cleaning products. However, inclusion of an antimicrobial claim in such cleaning products necessitates their registration as antimicrobial pesticides with the EPA. Currently, the EPA requires AMCPs to be tested in the Draize rabbit eye test in order to adequately characterize their ocular hazard potential.

# 2.0 Testing Strategies for Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products

## 2.1 AMCP Testing Strategy

The testing strategy (**Figure 2-1**) described in the AMCP BRD (**Annex I**) is based on the use of three *in vitro* test methods: BCOP, CM, and EO. Each test method includes decision criteria developed to correspond to the four categories of ocular irritation defined by the EPA classification system (i.e., EPA Category I, II, III, and IV [EPA 2003a]). These test methods use a variety of endpoints to predict ocular irritation potential.

The BCOP includes two primary endpoints (i.e., corneal opacity and permeability) that are measured quantitatively and used to calculate an *in vitro* irritancy score (IVIS).[3] An IVIS $\geq$75 = EPA Category I; IVIS $\geq$25 and <75 = EPA Category II; IVIS <25 = EPA Category III. The AMCP BRD does not propose decision criteria for EPA Category IV for the BCOP test method because the data points from EPA Category III and IV overlap and it is not possible to assign a cut-off value. Histopathology evaluation of the affected tissue is an optional endpoint for the BCOP test method. If a test substance produces an IVIS <75, further assessment using histopathology evaluation can determine whether it meets the criteria for classification as EPA Category I, II, or III.

The endpoint for the CM test method is the estimated concentration of a test substance needed to reduce the basal metabolic rate of L929 cells by 50% (the $MRD_{50}$). An $MRD_{50}$ <2 mg/mL = EPA Category I; $MRD_{50}$ $\geq$2 mg/mL and <80 mg/mL = EPA Category III; $MRD_{50}$ $\geq$80 mg/mL = EPA Category IV. The rationale for the use of L929 cells, a mouse fibroblast cell line, in the CM test method is provided in Section 2.2.1 of the AMCP BRD (**Annex I**). The AMCP BRD does not propose decision criteria for EPA Category II for the CM test method because the data points from EPA Category I and II overlap and it is not possible to assign a cut-off value.

The endpoint for the EO test method is the time needed to reduce cell viability by 50% ($ET_{50}$). An $ET_{50}$ <4 minutes = EPA Category I; $ET_{50}$ $\geq$4 minutes and <70 minutes = EPA Category III; $ET_{50}$ $\geq$70 minutes = EPA Category IV. The EO test method uses a proprietary tissue (i.e., EO tissue, MatTek Corporation, Ashland, MA) derived from normal human neonatal foreskin keratinocytes (see Section 2.2.2 of the AMCP BRD, **Annex I**). The keratinocytes are grown under standardized conditions to produce a highly uniform and reproducible cornea-like tissue. The AMCP BRD does not propose decision criteria for EPA Category II for the EO test method because only one EPA Category II substance is present in the database.

In the AMCP testing strategy as described in the AMCP BRD (**Figure 2-1**), the first test method used depends on knowledge of the chemical properties of the test substance. If the test substance is an oxidizer, which suggests that it will be an ocular corrosive or severe irritant, it is first tested in the BCOP test method. As noted above, test substances that produce an IVIS $\geq$75 would be classified as EPA Category I. If a test substance produces an IVIS <75, further assessment using histopathology evaluation can determine whether it meets the criteria for classification as EPA Category I, II, or III.

To determine whether the test substance is EPA Category III or IV, the test substance is subsequently tested in either the CM or EO test method to determine the final hazard category. Selection of the CM or EO test method depends on the water solubility of the test substance; water-soluble substances could be tested in either the CM test method or EO test method, but water-insoluble substances must be tested in the EO test method to determine their final hazard classification.

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[3] The *in vitro* irritancy score (IVIS) is calculated as the sum of the mean corrected opacity value (± standard deviation [SD]) and 15 times the mean corrected permeability value ($OD_{490}$ units ± SD).

## 2.2 Alternate AMCP Testing Strategy

Because none of the 228 substances has been tested in all three of the *in vitro* test methods included in the AMCP testing strategy, as well as concerns regarding the validation status of the LVET (ICCVAM 2009), which was used as the *in vivo* reference test method for all of the CM data, an alternate AMCP testing strategy (**Figure 2-2**) that includes only the BCOP and EO test methods was evaluated. In the alternate AMCP testing strategy, the BCOP test method would be used to identify EPA Category I or II substances and the EO test method would be used to identify EPA Category III or IV substances.

Testing in the alternate AMCP testing strategy (**Figure 2-2**) could proceed in one of two approaches: (1) test in the BCOP test method first and then in the EO test method or (2) test in the EO test method first and then in the BCOP test method. Using the first approach, the BCOP test method would classify all EPA Category I and II substances. All other substances would then be tested in the EO test method and classified as either EPA Category III or IV. Using the second approach, substances would first be tested in the EO test method, which would classify all EPA Category III and IV substances. All other substances would then be tested in the BCOP test method and classified as either EPA Category I or II.

**Figure 2-1 Combining the BCOP, CM, and EO Test Methods into a Testing Strategy: AMCP Testing Strategy**

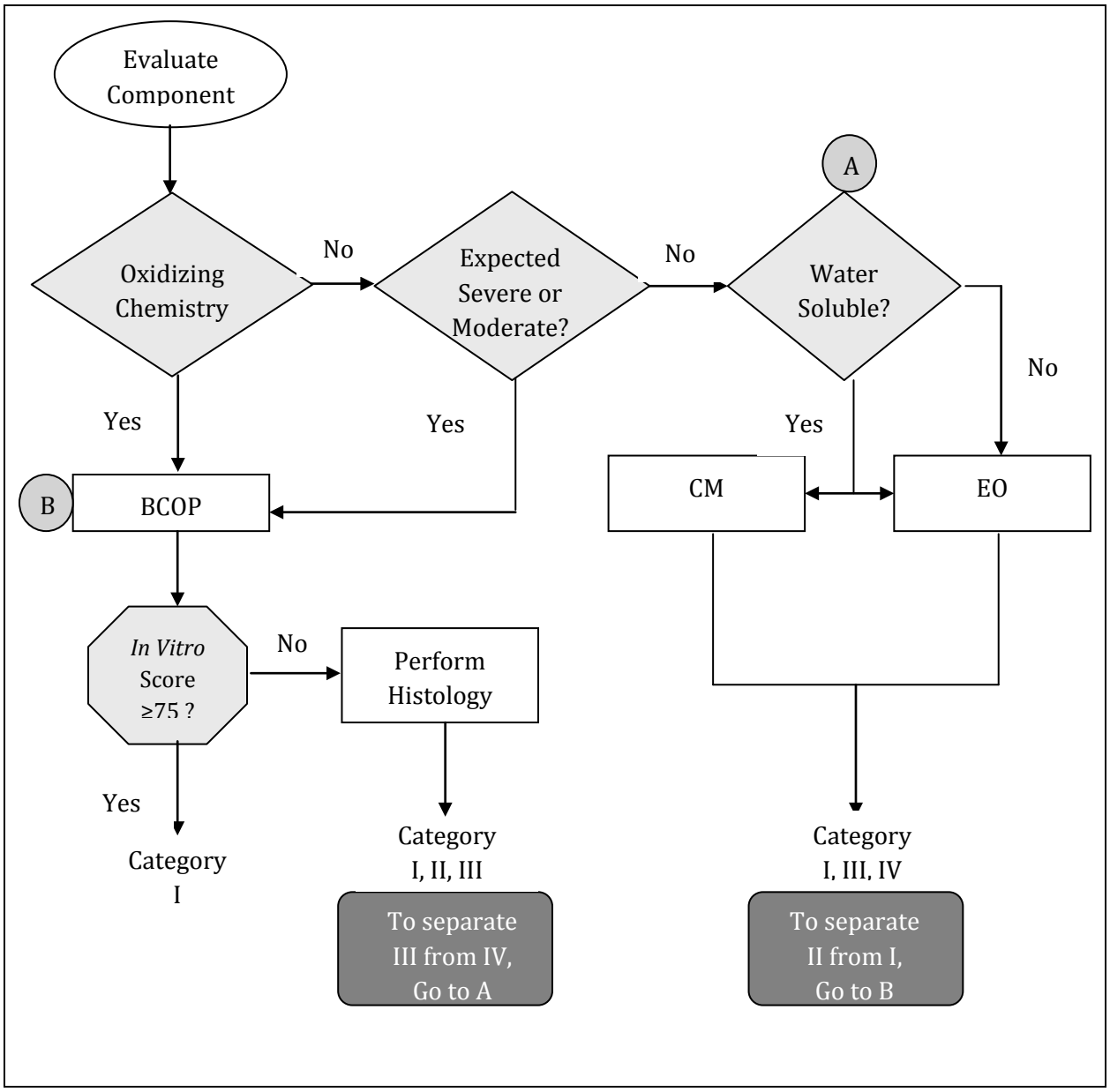
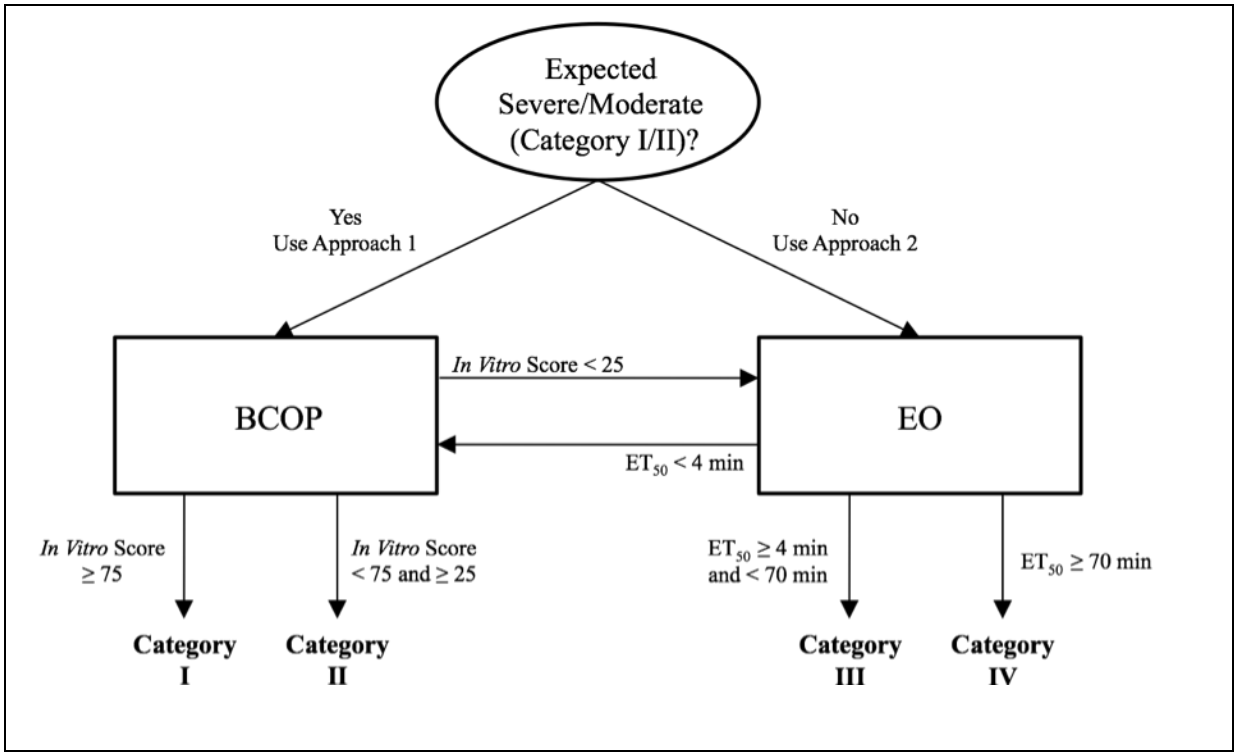


**Figure 2-2 Combining the BCOP and EO Test Methods into a Testing Strategy: Alternate AMCP Testing Strategy**



# 3.0 Substances Used for Validation of the Testing Strategies for EPA Classification of Antimicrobial Cleaning Products

## 3.1 Rationale for the Substances or Products Included in the AMCP Testing Strategy

A total of 228 substances were included in the validation database of the AMCP BRD (**Annex I**). It should be noted that, according to the submitter, "a minimum 28 of the materials are EPA registered anti-microbial cleaning products, with eight additional materials being in-use dilutions of concentrates which are EPA registered" (Rodger Curren, personal communication). Of these 228 substances, 68 substances were tested in the BCOP test method, 105 substances were tested in the CM test method, and 55 substances were tested in the EO test method. None of the 228 substances has been tested in all three of the *in vitro* test methods.

In the AMCP BRD, test substances were divided into "buckets" (i.e., chemical classes). The distribution of these chemical classes (solvents, oxidizers, surfactants, acids, bases, and others) by test method is presented in **Table 3-1**. Among the 68 substances tested in the BCOP test method, 18% (12/68) were solvents, 24% (16/68) were oxidizers, 33% (18/55) were surfactants, and 21% (14/68) were bases. Among the 105 substances tested in the CM test method, 17% (18/105) were solvents and 78% (82/105) were surfactants. Of 55 substances tested in the EO test method, 18% (10/55) were solvents, 24% (13/55) were oxidizers, 31% (17/55) were surfactants, and 20% (11/55) were bases.

**Table 3-1 Distribution of Product Categories Evaluated in the AMCP Testing Strategy**

| Product Categories | Number of Substances Tested Per Test Method | | | |
|---|---|---|---|---|
| | BCOP | Cytosensor Microphysiometer | EpiOcular | Total |
| Solvents | 12 | 18 | 10 | 39 |
| Oxidizers | 16 | 0 | 13 | 33 |
| Surfactants | 18 | 82 | 17 | 114 |
| Acids | 7 | 1 | 2 | 10 |
| Bases | 14 | 4 | 11 | 29 |
| Others | 1 | 0 | 2 | 3 |
| Total | 68 | 105 | 55 | 228 |

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability.

As reported in the AMCP BRD (**Annex I**), all 105 substances tested in the CM test method were tested in the LVET. No Draize rabbit eye test data were available for any of the substances tested in the CM test method. Of the 55 substances tested in the EO test method, 30 were tested in the Draize rabbit eye test and 25 were tested in the LVET. For the 68 substances tested in the BCOP test method, 58 were tested in the Draize rabbit eye test, 8 were tested in a nontraditional Draize rabbit eye test,[4] and 2 were tested in the LVET.

[4] The nontraditional Draize test data included seven substances tested with 30 ⌈L rather than the traditional 100 ⌈L instilled in the conjunctival sac of the rabbit and one substance that was tested as an aerosol sprayed directly on the cornea.

## 3.2 Rationale for the Substances or Products Included in the Alternate AMCP Testing Strategy

NICEATM requested additional ocular data on substances tested in either the BCOP or EO test methods. MatTek Corporation (Ashland, MA) provided additional EO data (for which BCOP and Draize rabbit eye test data were available). However, NICEATM determined that these data were generated using a different protocol or prediction model than described in the AMCP BRD (**Annex I**). No other data were found.

Of 29 substances tested in both the BCOP and EO test methods that were also tested in the Draize rabbit eye test, 28 substances met the criteria to assign an EPA hazard classification. The chemical categories for these 28 substances included five surfactants, two acids, ten alkalis, four oxidizers, six solvents, and one "other" (or nonspecified) as shown in **Table 3-2**. The composition of the 28 substances evaluated in the alternate AMCP testing strategy is provided in **Annex III**.

**Table 3-2 Distribution of Product Categories Evaluated in the Alternate AMCP Testing Strategy**

| Product Category | Number of Products Tested | *In Vivo* Draize Classification - EPA | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| Surfactant | 5 | 0 | 0 | 2 | 3 |
| Acid | 2 | 0 | 0 | 1 | 1 |
| Alkali | 10 | 9 | 1 | 0 | 0 |
| Oxidizer | 4 | 3 | 0 | 0 | 1 |
| Solvent | 6 | 2 | 0 | 1 | 3 |
| Other | 1 | 0 | 0 | 0 | 1 |
| Total | 28 | 14 | 1 | 4 | 9 |

Abbreviations: AMCP = antimicrobial cleaning product; EPA = U.S. Environmental Protection Agency.

## 4.0 *In Vivo* Reference Data

As reported in the AMCP BRD (**Annex I**), all 105 substances tested in the CM test method were tested in the LVET. No Draize rabbit eye test data were available for these substances. For the 55 substances tested in the EO test method, 25 were tested in the LVET and 30 were tested in the Draize rabbit eye test. Of those tested in the BCOP, 85% (58/68) were tested in the Draize rabbit eye test, 12% (8/68) were tested in a nontraditional Draize rabbit eye test,[5] and the remaining 3% (2/68) were tested in the LVET. The alternate AMCP testing strategy is based on the results for the 28 substances that were tested in both the BCOP and EO test methods, were also tested in the Draize rabbit eye test, and qualified for EPA hazard classification.

The Draize rabbit eye test (Draize et al. 1944) is the standard test method accepted by U.S. regulatory agencies such as the EPA for ocular irritation testing and for the classification and labeling of chemicals and products. The EPA (OPPTS 870.2400, EPA 1998) and the Organisation for Economic Co-operation and Development (OECD Test Guideline 405, OECD 2002) have published protocols describing the Draize rabbit eye test. The *in vivo* reference data are summarized in Section 4.2 of the AMCP BRD (**Annex I**), and the individual animal data are appended to that document.

The LVET is an *in vivo* rabbit eye test developed by Griffith et al. (1980) that differs from the Draize rabbit eye test by applying 10 μL (instead of 100 μL) of a test substance directly on the cornea (instead of the conjunctival sac). Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that of the Draize rabbit eye test. Background information on the LVET and comparison of the LVET to the Draize rabbit eye test is available in the ICCVAM test method evaluation report (ICCVAM 2010).

[5] The nontraditional Draize test data included seven substances tested with 30 μL rather than the traditional 100 μL instilled in the conjunctival sac of the rabbit and one aerosol test substance that was sprayed directly on the cornea.

# 5.0 Test Method Data and Results

## 5.1 AMCP Testing Strategy

The AMCP BRD (**Annex I**) includes, where available, the following specific information for each test substance: name, Chemical Abstracts Service Registry Number, physicochemical properties (e.g., purity, form tested), study reference, formulation ingredients, and chemical class. Test concentrations, individual and mean opacity scores, individual and mean permeability scores, $ET_{50}$ or $MRD_{50}$ values, and hazard classification information are also provided. If the source or purity of the test substance was missing, no attempt was made to identify it.

### 5.1.1 The Bovine Corneal Opacity and Permeability Test Method

Participating companies submitted BCOP data on 68 AMCPs generated using the ICCVAM-recommended BCOP protocol (ICCVAM 2006b). Of these substances, 66 had paired Draize rabbit eye test data (58 generated from the traditional Draize rabbit eye test protocol and 8 generated from a nontraditional Draize protocol, see **Section 3.1**). Two substances were tested in the LVET.

Supplemental BCOP data were included in the AMCP BRD (**Annex I**). These data were extracted from the BCOP BRD (ICCVAM 2006a).

### 5.1.2 The Cytosensor Microphysiometer Test Method

Participating companies submitted CM data on 105 unique AMCPs (with paired LVET data) generated using at least two different protocols. One protocol was based on the silicon microphysiometer (SM) test method, the predecessor of the CM test method, that used a 500-second exposure to L929 cells grown on a coverslip, compared to the CM protocol that used a 810-second exposure to cells grown on a Transwell™ membrane. An algorithm was derived and used to convert SM data to CM data. It should be noted that data analyses in the CM test method were based on 108 substances because three substances were tested twice, each with a different result.

Supplemental CM data were included in the AMCP BRD. The CTFA Phase III validation study provided data on surfactants and surfactant-based substances (n=25) with paired data from both the Draize rabbit eye test and the LVET (Gettings et al. 1996). CM data were also included from the EC/HO and COLIPA validation studies (Balls et al. 1995; Brantom et al. 1997).

### 5.1.3 The EpiOcular Test Method

Participating companies submitted EO data on 61 substances with formulations similar to those found in typical cleaning product formulations (**Annex I**). However, sufficient *in vivo* data to determine the EPA hazard classification were available for only 55 of these substances. Of these substances, 30 were tested in the Draize rabbit eye test data and 25 were tested in the LVET. Of the 30 substances tested in the Draize rabbit eye test, 29 qualified for EPA hazard classification (i.e., one substance producing a Draize score greater than 1 was not evaluated through day 21 as required by EPA).

Supplemental EO data were included in the AMCP BRD (**Annex I**). However, the EO protocol used in these studies differs significantly from the protocol being proposed in the AMCP BRD in that the test substance was diluted before testing; therefore, these studies were presented only as supporting information.

### 5.1.4 Combining the BCOP, CM, and EO Test Methods into a Testing Strategy: AMCP Testing Strategy

None of the 228 substances included in the AMCP BRD (**Annex I**) was tested in all three of the *in vitro* test methods in the AMCP testing strategy. Therefore, there are no data with which to characterize the actual performance of the AMCP testing strategy that includes the BCOP, CM, and EO test methods.

### 5.1.5 Combining the BCOP and EO Test Methods into a Testing Strategy: Alternate AMCP Testing Strategy

The evaluation of the alternate AMCP testing strategy was limited to the 28 substances that were tested in both the BCOP and EO test methods and also tested in the Draize rabbit eye test.

# 6.0 Test Method Accuracy

## 6.1 AMCP Testing Strategy

The AMCP BRD (**Annex I**) details the performance of each test method (i.e., BCOP, CM, and EO) included in the AMCP testing strategy. Performance is discussed according to the EPA (EPA 2003a) and GHS (UN 2007) classifications systems. Therefore, we only briefly summarize the performance of each test method. Additionally, because the results for the EPA and GHS classification systems are similar, only the EPA results are discussed. The data from the AMCP BRD are summarized in **Table 6-1**.

### 6.1.1 The Bovine Corneal Opacity and Permeability Test Method

Based on the validation database of 66 substances tested in both the BCOP test method and the Draize rabbit eye test, accuracy of the overall EPA classification (i.e., EPA Category I, II, III, and IV) was 55% (36/66) (**Table 6-1**). The BCOP test method correctly identified only 60% (3/5) of the EPA Category II and 50% (6/12) of the EPA Category III substances. However, the BCOP test method correctly identified 90% (27/30) of the EPA Category I substances. Among the three EPA Category I substances that the BCOP test method underpredicted as EPA Category II, two were oxidizers and one was a base. It should be noted that the base would have been correctly identified if the decision criteria were IVIS $\geq$55.1, as recommended in the BCOP BRD (ICCVAM 2006a), instead of IVIS $\geq$75 as proposed in the AMCP BRD (**Annex I**). However, such a change in decision criteria would also result in two EPA Category II substances (one oxidizer and one acid) and one EPA Category III substance (a base) being overpredicted as EPA Category I.

Among the EPA Category II substances that were incorrectly identified by the BCOP test method, one (a base) was underclassified as EPA Category III, and one (an oxidizer) was overclassified as EPA Category I. All six EPA Category III substances that were incorrectly identified by the BCOP test method were overclassified as either EPA Category I (two oxidizers and one base) or EPA Category II (one solvent, one base, and one surfactant). Because decision criteria for the BCOP test method are not proposed in the AMCP BRD for EPA Category IV, all 19 substances were overpredicted: two as EPA Category II (both solvents) and 17 as EPA Category III (8 surfactants, 3 solvents, 3 acids, one base, one oxidizer, and one "other").

To assess the use of histopathology evaluation, BCOP test method data with histopathology evaluation were compared to BCOP test method data only. Data were available for 17 substances that had BCOP data with histopathology evaluation. As noted in **Table 6-2**, the overall accuracy for EPA hazard classifications (i.e., EPA Category I, II, III, and IV) was reduced from 41% (7/17) to 35% (6/17) with histopathology evaluation. Using histopathology evaluation with the BCOP test method removed one of the EPA Category I false negatives, but added three EPA Category II false positives.

### 6.1.2 The Cytosensor Microphysiometer Test Method

Based on the database of 108 substances tested in both the CM test method and the LVET (**Table 6-1**), accuracy of the overall EPA classification was 30% (32/108). It should be noted that the database consisted of 105 unique substances because three substances were tested twice. The CM test method overclassified the majority of EPA Category II, III, and IV substances included in the database (100% [11/11] of the EPA Category II substances, 67% [40/60] of the EPA Category III substances, and 89% [25/28] of the EPA Category IV substances). Among the 25 EPA Category IV substances that were overclassified, the CM test method classified 16% (4/25, all surfactants) as EPA Category I and 84% (21/25, 6 solvents, 2 bases, and 13 surfactants) as EPA Category III. Because decision criteria for the CM test method are not proposed in the AMCP BRD for EPA Category II, all

EPA Category II or III substances that were overclassified by the CM test method were classified as EPA Category I. All but one of the 40 EPA Category III substances that were overclassified by the CM test method were surfactants. The remaining one was a solvent. All 11 EPA Category II substances that were overclassified by the CM test method were surfactants. All nine of the EPA Category I substances (all surfactants) were correctly identified. None of the irritant categories (i.e., EPA Category I, II, or III) were underpredicted by the CM test method.

**Table 6-1 Performance of AMCPs in the Bovine Corneal Opacity and Permeability, Cytosensor Microphysiometer, and EpiOcular Test Methods Compared to the Draize Rabbit Eye Test or the Low Volume Eye Test as Reported in the AMCP BRD Using the EPA Classification System**

| ***In Vitro* Test Method** | ***In Vivo* Test Method** | **Overall Classification** | **Performance of the *In Vitro* Test Method Compared to the *In Vivo* Reference Test Method Using the EPA Classification System** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **I** | | **II** | | | **III** | | | **IV** | |
| | | | **Actual** | **Under** | **Over** | **Actual** | **Under** | **Over** | **Actual** | **Under** | **Over** | **Actual** |
| BCOP[1] | Draize | 55% (36/66) | 90% (27/30) | 10% (3/30) | 20% (1/5) | 60% (3/5) | 20% (1/5) | 50% (6/12) | 50% (6/12) | 0% (0/12) | 100% (19/19) | 0% (0/19) |
| CM[2] | LVET | 30% (32/108) | 100% (9/9) | 0% (0/9) | 100% (11/11) | 0% (0/11) | 0% (0/11) | 67% (40/60) | 33% (20/60) | 0% (0/60) | 89% (25/28) | 11% (3/28) |
| EO[3] | Draize | 76% (22/29) | 100% (15/15) | 0% (0/15) | 0% (0/1) | 0% (0/1) | 100% (1/1) | 25% (1/4) | 75% (3/4) | 0% (0/4) | 56% (5/9) | 44% (4/9) |
| EO[4] | LVET | 44% (11/25 | 100% (3/3) | 0% (0/3) | 100% (1/1) | 0% (0/1) | 0% (0/1) | 33% (4/12) | 67% (8/12) | 0% (0/12) | 100% (9/9) | 0% (0/9) |

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; CM = Cytosensor Microphysiometer; EO = EpiOcular; EPA = U.S. Environmental Protection Agency; ET50 = estimated time to decrease keratinocyte viability in the EO test method by 50%; IVIS = in vitro irritancy score; LVET = low volume eye test; MRD50 = concentration of test substance that decreases the metabolic rate by 50% determined by a plot of the concentration-response curve.

[1] Classification of the BCOP data was based on IVIS ≥75 = EPA Category I; IVIS ≥25 and <75 = EPA Category II; IVIS <25 = EPA Category III. The BCOP test method was not proposed to identify EPA Category IV. All BCOP classifications, including high-solvent substances, used a 10-minute exposure time. The database comprised 66 substances tested in both the BCOP test method and the Draize rabbit eye test.

[2] Classification of the CM data was based on MRD50 <2 mg/mL = EPA Category I; MRD50 ≥2mg/mL and <80 mg/mL = EPA Category III; MRD50 ≥80 mg/mL = EPA Category IV. The CM test method was not proposed to identify EPA Category II. The database consisted of 108 substances tested in both the CM test method and in the LVET (105 different substances because three substances were tested twice).

[3] Classification of the EO data was based on ET50 <4 min = EPA Category I; ET50 ≥4 min and <70 min = EPA Category III; ET50 ≥70 min = EPA Category IV. The EO test method was not proposed to identify EPA Category II. The database consisted of 29 substances tested in both the EO test method and the Draize rabbit eye test that qualified for EPA hazard classification (i.e., one substance producing a Draize score greater than 1 was not evaluated through day 21 as required by EPA).

[4] Classification of the EO data was based on ET50 <4 min = EPA Category I; ET50 ≥4 min and <70 min = EPA Category III; ET50 ≥70 min = EPA Category IV. The EO test method was not proposed to identify Category II. The database consisted of 25 substances tested in both the EO test method and the LVET.

**Table 6-2 Comparison of the BCOP Test Method and the BCOP Test Method Using Histopathology Evaluation**

| EPA | Overall Classification | Draize | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | II | | | III | | | IV[1] | |
| | | Actual | Under | Over | Actual | Under | Over | Actual | Under | Over | Actual |
| BCOP[2] Only | 41% (7/17) | 50% (3/6) | 50% (3/6) | 0% (0/4) | 75% (3/4) | 25% (1/4) | 75% (3/4) | 25% (1/4) | 0% (0/4) | 100% (3/3) | 0% (0/3) |
| BCOP[2] with Histology | 35% (6/17) | 67% (4/6) | 33% (2/6) | 75% (3/4) | 25% (1/4) | 0% (0/4) | 75% (3/4) | 25% (1/4) | 0% (0/4) | 100% (3/3) | 0% (0/3) |

Abbreviations: BCOP = bovine corneal opacity and permeability.

[1] The BCOP test method decision criteria do not propose to identify EPA Category IV substances.

[2] The BCOP test method was based on the use of AMCP decision criteria with a cutoff for corrosives or severe irritants of ≥75 tested with a 10-minute exposure time.

### 6.1.3 The EpiOcular Test Method

Among the 55 substances tested in the EO test method (**Table 6-1**), 30 were also tested in the Draize rabbit eye test and 25 were tested in the LVET. Of the 30 substances tested in the Draize rabbit eye test, 29 qualified for EPA hazard classification (i.e., one substance producing a Draize score greater than 1 was not evaluated through day 21 as required by EPA). For these 29 substances, accuracy of the overall EPA classification was 76% (22/29). Among the four EPA Category III substances, the EO test method correctly identified 75% (3/4). The one substance incorrectly identified (a base) was overclassified as EPA Category I. Among the nine EPA Category IV substances, 44% (4/9) were correctly identified. Four of the five incorrectly identified substances were overclassified as EPA Category III (two solvents, one acid, and one surfactant). The remaining substance (a surfactant) was overclassified as EPA Category I. All of the EPA Category I substances (15/15, including nine bases, three oxidizers, two solvents, and one "other") were correctly identified.

The EO test method correctly classified 44% (11/25) of the 25 substances tested in both the EO test method and the LVET (**Table 6-1**). Among the 12 EPA Category III substances, (67% (8/12) were correctly identified by the EO test method. The four substances incorrectly identified (two surfactants and two oxidizers) were overclassified as EPA Category I. None of the nine EPA Category IV substances were correctly identified: 44% (4/9, including three surfactants and one solvent) were overclassified as EPA Category III, and 56% (5/9, including three oxidizers and two solvents) were overclassified as EPA Category I. The EO test method correctly identified all three of the EPA Category I substances (two oxidizers and one surfactant).

### 6.1.4 Combining the BCOP, CM, and EO Test Methods into a Testing Strategy: AMCP Testing Strategy

The performance of each test method included in the AMCP testing strategy is summarized in **Table 6-1**. None of the 228 substances included in the AMCP BRD was tested in all three of the *in vitro* test methods proposed for the AMCP testing strategy. Therefore, no data are available with which to characterize the actual performance of the AMCP testing strategy that includes the BCOP, CM, and EO test methods.

## 6.2 Combining the BCOP and EO Test Methods into a Testing Strategy: Alternate AMCP Testing Strategy

The performance of the alternate AMCP testing strategy was based on the 28 substances that were tested in both the BCOP and EO test methods with Draize rabbit eye test data (**Annex IV**). As noted in **Section 2.0**, these data were evaluated based on two approaches: (1) test in the BCOP test method first and then in the EO test method, or (2) test in the EO test method first and then in the BCOP test method. Using the first approach, the BCOP test method would first classify all EPA Category I or II results. All other substances would then be tested in the EO test method and classified as either EPA Category III or IV. Using the second approach, the EO test method would first classify all EPA Category III or IV results. All other substances would then be tested in the BCOP test method and classified as either EPA Category I or II.

Regardless of which approach was used, the performance of the alternate AMCP testing strategy was the same (see **Sections 6.2.1** and **6.2.2**). The overall correct classification of the BCOP data using either the decision criteria in the AMCP BRD (**Annex I**) (IIVS ≥75 to assign EPA Category 1) or in the BCOP BRD (ICCVAM 2006a) (IIVS ≥55 to assign EPA Category I) yielded identical results. All BCOP classifications, including high-solvent substances, used a 10-minute exposure time. When using 3-minute data for high solvents, the overall classification is 74% (17/23). Five high-solvent substances did not have 3-minute data and, therefore, cannot be considered in this analysis.

### 6.2.1 Approach 1: Test in the BCOP Test Method First and then in the EO Test Method

Using Approach 1 and either the ≥55.1 or ≥75 cutoff value to identify EPA Category I substances, the overall correct classification was 79% (22/28) (**Table 6-3**). The boxes in **Table 6-3** represent the correct calls for the BCOP test method (bolded numbers) and for the EO test method (numbers in parentheses). All of the substances classified as EPA Category I by the Draize rabbit eye test were correctly identified by the alternate AMCP testing strategy using Approach 1 (100% [14/14]). The EO test method correctly predicted all (100%; 4/4) of the EPA Category III substances and 44% (4/9) of the EPA Category IV substances. Thus, the EO test method overpredicted 56% (5/9) as EPA Category III.

### 6.2.2 Approach 2: Test in the EO Test Method First and then in the BCOP Test Method

Using Approach 2 and either the ≥55.1 or ≥75 cutoff value to identify EPA Category I substances, the overall correct classification was 79% (22/28) (**Table 6-4**). The boxes in **Table 6-4** represent the correct calls for the BCOP test method (bolded numbers) and for the EO test method (numbers in parentheses). The EO test method correctly identified all (100%; 4/4) of the EPA Category III substances and 44% (4/9) of the EPA Category IV substances. Five EPA Category IV substances (56% [5/9]) were overclassified by the EO test method as EPA Category III. All of the substances classified as EPA Category I by the Draize rabbit eye test were correctly identified by the alternate AMCP testing strategy using Approach 2 (100% [14/14]). The BCOP test method overpredicted one EPA Category IV substance as EPA Category II.

**Table 6-3 Performance of AMCPs Tested in Both the BCOP and EO Test Methods Using Approach 1[1]**

| EPA | | Classification (BCOP→EO)[2] Using Approach 1 | | | | |
|---|---|---|---|---|---|---|
| | | **I** | **II** | **III** | **IV** | **Totals** |
| Draize Classification | I | **14** (0) | **0** (0) | **0** (0) | **0** (0) | 14 |
| | II | **0** (0) | **0** (0) | **0** (1) | **0** (0) | 1 |
| | III | **0** (0) | **0** (0) | **0** (4) | **0** (0) | 4 |
| | IV | **0** (1) | **1** (0) | **0** (3) | **0** (4) | 9 |
| | Total | 14 (1) | 1 (0) | 0 (8) | 0 (4) | 28 |

| EPA | Overall Classification | Draize | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **I** | | **II** | | | **III** | | | **IV** | |
| | | **Actual** | **Under** | **Over** | **Actual** | **Under** | **Over** | **Actual** | **Under** | **Over** | **Actual** |
| Approach to Identify Ocular Corrosives and Severe Irritants | 79% (22/28) | 100% (14/14) | 0% (0/14) | 0% (0/1) | 0% (0/1) | 100% (1/1) | 0% (0/4) | 100% (4/4) | 0% (0/4) | 56% (5/9) | 44% (4/9) |

Abbreviations: BCOP = bovine corneal opacity and permeability; EO = EpiOcular; EPA = U.S. Environmental Protection Agency.

[1] Boldface numbers represent the classification by the BCOP test method, and numbers in parentheses represent the classification by the EO test method when using the alternate AMCP testing strategy.

[2] In the alternate AMCP testing strategy, the BCOP test method is only intended to identify EPA Category I or II substances, and the EO test method is intended to identify only EPA Category III or IV substances.

**Table 6-4 Performance of AMCPs Tested in Both the BCOP and EO Test Methods Using Approach 2[1]**

| EPA | | Classification (EO→BCOP)[2] Approach 2 | | | | |
|---|---|---|---|---|---|---|
| | | **I** | **II** | **III** | **IV** | **Totals** |
| Draize Classification | I | **14 (0)** | **0 (0)** | **0 (0)** | **0 (0)** | 14 |
| | II | **0 (0)** | **0 (0)** | **0 (1)** | **0 (0)** | 1 |
| | III | **0 (0)** | **0 (0)** | **0 (4)** | **0 (0)** | 4 |
| | IV | **0 (0)** | **0 (0)** | **1 (4)** | **0 (4)** | 9 |
| | Totals | **14 (1)** | **0 (0)** | **1 (9)** | **0 (4)** | 28 |

| EPA | Overall Classification | Draize | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **I** | | **II** | | | **III** | | | **IV** | |
| | | **Actual** | **Under** | **Over** | **Actual** | **Under** | **Over** | **Actual** | **Under** | **Over** | **Actual** |
| Approach to Identify Category IV | 79% (22/28) | 100% (14/14) | 0% (0/14) | 0% (0/1) | 0% (0/1) | 100% (1/1) | 0% (0/4) | 100% (4/4) | 0% (0/4) | 56% (5/9) | 44% (4/9) |

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; EO = EpiOcular; EPA = U.S. Environmental Protection Agency.

[1] Boldface numbers represent the classification by the BCOP test method, and numbers in parentheses represent the classification by the EO test method when using the alternate AMCP testing strategy.

[2] In the alternate AMCP testing strategy, the BCOP test method is only intended to identify EPA Category I or II substances, and the EO test method is intended to identify only EPA Category III or IV substances.

# 7.0 Reliability of the Test Methods Used in the Antimicrobial Cleaning Product Testing Strategy

An assessment of test method reliability is essential to any evaluation of the performance of an alternative test method (ICCVAM 2003). NICEATM assessed test method reliability by analyzing the following:

- Intralaboratory repeatability: multiple runs of a substance in a test method conducted by a single laboratory over a short period of time
- Intralaboratory reproducibility: multiple runs of a substance in a test method conducted by a single laboratory over an extended period of time under similar conditions using identical protocols
- Interlaboratory reproducibility: multiple runs of a substance in a test method conducted among several laboratories over an extended period of time under similar conditions using identical protocols

While some measures of repeatability and reproducibility were conducted using data presented in the AMCP BRD (**Annex I**), insufficient data were available to accurately determine the reliability of the test methods. Additional data on the reliability of each test method were provided by the Institute for In Vitro Sciences, Inc. as an AMCP BRD Supplement (**Annex II**). Data from the BCOP BRD (ICCVAM 2006a) were also used to establish reliability of the BCOP test method.

## 7.1 The Bovine Corneal Opacity and Permeability Test Method

### 7.1.1 Intralaboratory Repeatability

Intralaboratory repeatability for the BCOP test method was quantitatively determined for 67 AMCPs (four substances have repeat tests) as the mean %CV for opacity, permeability, and IVIS in the AMCP BRD (**Annex I**). Because %CVs are significantly affected by scores in the very low range, the mean %CVs from materials with an IVIS $\leq$ 10 (arbitrarily set in the AMCP BRD) were not considered in the overall mean %CV calculations. The overall mean %CV for opacity, permeability, and IVIS was 21%, 25%, and 18%, respectively.

These test substances, tested in a total of 75 runs, were also qualitatively evaluated for their concordance using the EPA (EPA 2003a) and GHS (UN 2007) classification systems (**Annex II**). For the EPA and GHS classification systems, there was 100% agreement for 63 of the 75 runs (84%), 67% agreement for 11 of the 75 runs (15%), and 60% agreement for 1 of the 75 runs (1%). Of the 12 runs that did not have 100% agreement, seven had reactive chemistries, two were alkalis, two were surfactants, and one was an acid.

Intralaboratory repeatability for the BCOP test method was quantitatively determined for non-AMCPs predicted as severe or ocular corrosives in the BCOP test method in three studies (16–52 substances) (ICCVAM 2006a). The mean %CV for IVIS ranged from 39% to 71%.

### 7.1.2 Intralaboratory Reproducibility

Intralaboratory reproducibility for the BCOP test method was quantitatively determined for AMCPs (n=5) as the mean %CV for IVIS. For these five substances (2–6 experiments), the mean %CV for IVIS was 20% (see Section 7.3 of the AMCP BRD, **Annex I**).

These test substances were also qualitatively evaluated for their concordance using the EPA (EPA 2003a) and GHS (UN 2007) classification systems (see Section 3.2 of the AMCP BRD Supplement, **Annex II**). Using either the EPA or GHS classification systems, there was 100% agreement for the five test substances.

Intralaboratory reproducibility for the BCOP test method has been quantitatively determined for non-AMCPs predicted as severe or ocular corrosives in the BCOP test method in two studies (ICCVAM 2006a). In one study composed of 25 surfactant-based personal care cleaning formulations, the mean %CV for permeability values was 33%. In the second study (n=16), the mean %CV for IVIS ranged from 13% to 15%.

### 7.1.3 Interlaboratory Reproducibility

Interlaboratory reproducibility the BCOP test method cannot be determined specifically for the AMCPs presented in the AMCP BRD (**Annex I**) because only one laboratory conducted the testing.

Interlaboratory reproducibility for the BCOP test method has been quantitatively determined for non-AMCPs predicted as severe or ocular corrosives in the BCOP test method in three studies (3-12 laboratories each) (ICCVAM 2006a). The mean %CV for IVIS ranged from 25% to 36%. These test substances were also qualitatively evaluated (ICCVAM 2006a) for their concordance using the EPA (EPA 2003a), GHS (UN 2007), and EU (EU 2001) classification systems.

## 7.2 The Cytosensor Microphysiometer Test Method

### 7.2.1 Intralaboratory Repeatability

Reliability for the CM test method could not be evaluated specifically for AMCPs due to insufficient data. However, quantitative evaluations of reliability were conducted based on non-AMCPs tested in the CM test method (**Annexes I** and **II**).

Intralaboratory repeatability for the CM test method was quantitatively evaluated for non-AMCPs in seven studies (n=1–35 test substances per study) (**Annexes I** and **II**). The mean %CV for $MRD_{50}$ values for all materials tested, including surfactant and nonsurfactant materials, ranged from 6% to 25%.

### 7.2.2 Intralaboratory Reproducibility

Intralaboratory reproducibility for the CM test method was quantitatively determined for non-AMCPs in one laboratory (16 substances) (**Annex I**). The mean %CV for $MRD_{50}$ values for all materials tested, including surfactant and nonsurfactant materials, was 25%.

### 7.2.3 Interlaboratory Reproducibility

Interlaboratory reproducibility for the CM test method was quantitatively determined for non-AMCPs in two studies (2–4 laboratories each) (**Annexes I** and **II**). The mean %CV for $MRD_{50}$ values for all materials tested, including surfactant and nonsurfactant materials, ranged from 17% to 51%. Nonsurfactant materials had a higher mean %CV in each study.

## 7.3 The EpiOcular Test Method

### 7.3.1 Intralaboratory Repeatability

Intralaboratory repeatability for the EO test method was quantitatively determined specifically for a subset of AMCPs (n=15) presented in the AMCP BRD (**Annex I**). The mean %CV for $ET_{50}$ values ranged from 0% to 62%.

Qualitative analyses were conducted with three AMCPs that were tested more than once at the Institute for In Vitro Sciences, Inc. to evaluate the extent of agreement using the EPA (EPA 2003a) or GHS (UN 2007) hazard classification system (**Annex II**). There was 100% agreement for all three AMCPs for both EPA and GHS classification systems.

### 7.3.2 Intralaboratory Reproducibility

Intralaboratory reproducibility for the EO test method was also quantitatively determined from repeat testing of a single substance (0.3% Triton® X-100). Data were presented as combined data from MatTek Corporation and the Institute for In Vitro Sciences, Inc. (9-year period) and from the Institute for In Vitro Sciences, Inc., only (8-year period). The mean %CV for $ET_{50}$ values was 21% and 22%, respectively.

### 7.3.3 Interlaboratory Reproducibility

Interlaboratory reproducibility for the EO test method cannot be determined specifically for the AMCPs presented in the AMCP BRD (**Annex I**) because only one laboratory conducted the testing. However, interlaboratory reproducibility for the EO test method was quantitatively determined for non-AMCPs in a two-phase validation study for surfactants and surfactant-containing products, which is summarized in the AMCP BRD (**Annex I**). Based on the validation study, the mean %CVs ranged from 12% to 18%. However, it should be noted that this evaluation of reproducibility is based on an EO protocol that uses relative percent viability to assign an irritancy classification (i.e., irritant vs. nonirritant) and not on a calculated $ET_{50}$ value to predict the ocular hazard classification category (i.e., EPA Category I, II, III, and IV). The latter is the protocol included in the AMCP BRD.

These test substances were also qualitatively evaluated for their concordance using the EPA (EPA 2003a) and GHS (UN 2007) classification systems (**Annex II**). Using either the EPA or GHS classification systems in Phase II of the validation study, there was 100% agreement for 74% (14/19) of the substances, 75% agreement for 11% (2/19) of the substances, and 50% agreement for 16% (3/19) of the substances among four laboratories. In Phase III of the validation study using the EPA or GHS classification systems, there was 100% agreement for 94% (51/54) of the substances and 0% agreement for 6% (3/54) of the substances in two laboratories.

# 8.0 Data Quality: Antimicrobial Cleaning Product Background Review Document

## 8.1 Adherence to National and International Good Laboratory Practice Guidelines

The extent to which the studies included in the AMCP BRD (**Annex I**) complied with national and international Good Laboratory Practice (GLP) guidelines (OECD 1998; EPA 2003b, 2003c; FDA 2003) is based on the information provided in the AMCP BRD. While it could not be ascertained whether all of the *in vitro* data provided in the AMCP BRD were GLP compliant, the data determined to be GLP compliant were noted in the spreadsheets that contain the study data. All of the laboratories that contributed data for these studies have experience conducting GLP-compliant studies. All of the new data generated for the studies in the AMCP BRD were collected according to GLP guidelines.

## 8.2 Data Quality Audits

Formal assessments of data quality, such as quality assurance audits, generally involve a systematic and critical comparison of the data provided in the study report to the laboratory records generated during the study. No data quality audits were specifically conducted in the preparation of the AMCP BRD (**Annex I**). However, the studies conducted according to GLP guidelines would have included such an audit.

## 8.3 Impact of Deviations from GLP Guidelines

The impact of deviations from GLP guidelines cannot be evaluated because no information on data quality audits was obtained.

## 8.4 Availability of Laboratory Notebooks or Other Records

The original study notebooks, final reports, and other background information were available for the majority of the studies reported in the AMCP BRD (**Annex I**). The individual companies that contributed data to the AMCP BRD consider these materials confidential and requested that they not be associated with any particular product. Thus, the study materials are available for inspection, if requested by NICEATM or the EPA, with company identifiers removed to ensure compliance with this request.

# 9.0 Other Scientific Reports and Reviews

## 9.1 The Bovine Corneal Opacity and Permeability Test Method

For the BCOP test method, NICEATM identified four studies that had been published since the previous evaluation of the BCOP test method for the identification of ocular corrosives and severe irritants (ICCVAM 2006a): Debbasch et al. 2005; Van Goethem et al. 2006; Cater and Harbell 2006; and Cater and Harbell 2008. However, none of these publications included Draize rabbit eye test data; therefore, these studies were not added to the database.

### 9.1.1 Debbasch et al. (2005)

Twelve makeup removers were tested in both the BCOP test method and in a clinical in-use test under ophthalmological control. The undiluted test product (750 μL) was pipetted onto the corneas and exposure was conducted for 4 hours. Corneal opacity was determined using an adapted spectrophotometer and barrier disruption by fluorescein update using $OD_{490}$ mm. *In vitro* scores were classified according to Gautheron et al. (1994) and Harbell and Curren (1998).

### 9.1.2 Cater and Harbell (2006)

Surfactant-based "rinse-off" personal care formulations were tested in the BCOP test method using slight modifications of the BCOP test method protocol reported by Sina et al. (1995). Corneas were exposed to the test substances (750 μL) for 10, 30, or 60 minutes either undiluted or diluted in deionized water. Corneas were evaluated for opacity, fluorescein uptake, and histological alterations.

### 9.1.3 Van Goethem et al. (2006)

Van Goethem et al. tested 20 substances in the BCOP test method (7 compounds classified as GHS Not Classified and 13 GHS Category 1). Vanparys et al. (1993) and Gautheron et al. (1994) previously published these results, which were included in the BCOP BRD (ICCVAM 2006a).

### 9.1.4 Cater and Harbell (2008)

The BCOP test method was used on four commercial and one unregistered body wash. The purpose was to determine if the BCOP test method could be used as a prediction model for relative ranking of human eye responses to surfactant-based formulations under conditions of a standard human eye sting test. Test articles were prepared as 25% solutions in deionized water; 750 μL was applied to the corneas for a 30-minute exposure. Following exposure, opacity and fluorescein uptake were determined.

## 9.2 The Cytosensor Microphysiometer Test Method

A BRD for the CM test method,[6] which includes a comprehensive review of all available data, was submitted to the European Centre for the Validation of Alternative Methods (ECVAM) for review of its validation status in Europe.

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[6] A redacted version of the ECVAM CM BRD is available on the NICEATM-ICCVAM website. The main body of the document is available at http://iccvam.niehs.nih.gov/methods/ocutox/CM/ECVAM-CMBRD-Aug08redact.pdf and the annexes to the document are available at http://iccvam.niehs.nih.gov/methods/ocutox/CM/CMBRD-AnnexesAug08redact.pdf.

## 9.3 The EpiOcular Test Method

A BRD for the EO test method, which includes a comprehensive review of all available data, was submitted to ECVAM for review of its validation status in Europe. To date, this document has not been made available to the public.

# 10.0 Animal Welfare Considerations

## 10.1 How the AMCP Testing Strategy and *In Vitro* Methods will Refine, Reduce, or Replace Animal Use

Draize rabbit eye test data are currently used to classify and label AMCPs. The AMCP testing strategy described in the AMCP BRD (**Annex I**) or the alternate AMCP testing strategy would provide a non-animal approach to EPA classification and labeling of AMCPs and could thereby eliminate the use of rabbits for this type of testing.

## 10.2 Requirements for the Use of Animals

The EPA currently requires a Draize rabbit eye test for classification and labeling of AMCPs. The Draize rabbit eye test protocol is provided in the EPA Health Effects Test Guideline (OPPTS 87.2440; EPA 1998) and in OECD Test Guideline 405 (OECD 2002). The Draize rabbit eye test requires only one animal if the test substance is shown to be corrosive or a severe (irreversible) eye irritant. It requires three animals per test substance for nonsevere irritants or nonirritants. These animals are in addition to similar sets of animals for both the positive and negative control groups within a study of multiple test substances. More animals may be required if the EPA classification results are equivocal.

The BCOP test method uses ocular tissue obtained from animals that are being procured for food. Cattle are not subject to pain and suffering during the harvest of corneal tissue, because it is obtained post mortem and would otherwise be discarded by the meatpacker.

No animals are used for the CM test method, except for the mice used to establish the original mouse fibroblast cell line.

The EO test method uses a three-dimensional corneal construct generated with primary human keratinocytes. These cells are obtained during routine surgical procedures, and their procurement to initiate a cell culture does not subject the donor to any pain or suffering.

# 11.0 Practical Considerations

Several issues in addition to performance evaluations must be considered when assessing the practicality of an alternative test method in comparison to the existing test method:

- Laboratory equipment and supplies needed to conduct the alternative test method
- Level of personnel training
- Labor costs
- Time required to complete the test method

The time, personnel cost, and effort required to conduct the proposed test method must be considered reasonable in comparison to those of the test method it is intended to replace.

## 11.1 Transferability of the Test Methods Included in the AMCP Testing Strategy

Test method transferability addresses the ability of a test method to be performed accurately and reliably by multiple laboratories (ICCVAM 2003), including those experienced in the particular type of procedure and those with less or no experience in the particular procedure. The degree of transferability of a test method can be evaluated based on interlaboratory reproducibility (see **Section 7.0**).

One important consideration regarding the transferability of the CM test method is that the instrument has been discontinued. Therefore, a user would have to obtain a used instrument or have one manufactured before testing.

## 11.2 Training Considerations

The AMCP BRD (**Annex I**) details the level of training and expertise needed to conduct the test methods used in the AMCP testing strategy and the training requirements needed to demonstrate proficiency based on the ICCVAM test method submission guidelines (ICCVAM 2003).

## 11.3 Cost Considerations

At the present time, the cost of running a GLP-compliant Draize rabbit eye test ranges from $1200 to $14,500 depending on the number of days the animals have to remain on the study (i.e., 21 days or less). A GLP-compliant BCOP test method will cost approximately $1500 for a single test substance. The cost of performing the BCOP test method is approximately doubled when histopathology evaluation is included. A GLP-compliant CM test method will cost approximately $2000 for each of a minimum of two test substances. A GLP-compliant EO test method will cost approximately $3000 for a single test substance. For each of these *in vitro* test methods, the cost per sample is significantly reduced when multiple substances are run concurrently.

## 11.4 Time Considerations

The Draize rabbit eye test or the LVET could require up to 21 days, in addition to several pretest days to acclimatize the animals. The BCOP test method can be completed in one day, but histopathology evaluation may require an additional four weeks. The CM test method, including multiple runs of the test substance, can be completed in a one day. The EO test method can be performed in two days, although it may take several weeks to acquire the tissue.

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## 13.0 Glossary[7]

**Accuracy:**[8] (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of *relevance*. The term is often used interchangeably with *concordance* (see also *two-by-two table*). Accuracy is highly dependent on the prevalence of positives in the population being examined.

**Antimicrobial cleaning product (AMCP):** Commercially available household cleaning products are regulated by the CPSC. However, when an antimicrobial claim is made, these products must be registered as pesticides with the EPA.

**Blepharitis:** Inflammation of the eyelid.

**Chemosis:** A form of eye irritation in which the membranes that line the eyelids and surface of the eye (*conjunctivae*) become swollen.

**Classification system:** An arrangement of quantified results or data into groups or categories according to previously established criteria.

**Coded substances:** Substances labeled by code rather than name so that they can be tested and evaluated without knowledge of their identity or anticipation of test results. Coded substances are used to avoid intentional or unintentional bias when evaluating laboratory or test method performance.

**Coefficient of variation:** A statistical representation of the precision of a test. It is expressed as a percentage and is calculated as follows:

$$\left(\frac{standard\ \ deviation}{mean}\right) \times 100\%$$

**Concordance:**[8] The proportion of all substances tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of *relevance*. The term is often used interchangeably with *accuracy* (see also *two-by-two table*). Concordance is highly dependent on the prevalence of positives in the population being examined.

**Conjunctiva:** The mucous membrane that lines the inner surfaces of the eyelids and folds back to cover the front surface of the eyeball, except for the central clear portion of the outer eye (the cornea). The conjunctiva is composed of three sections: palpebral conjunctiva, bulbar conjunctiva, and fornix.

**Conjunctival sac:** The space located between the eyelid and the conjunctiva-covered eyeball. Substances are instilled into the sac to conduct an *in vivo* eye test.

**Cornea:** The transparent part of the coat of the eyeball that covers the iris and pupil and admits light to the interior.

**Corneal opacity:** Measurement of the extent of opaqueness of the cornea following exposure to a test substance. Increased corneal opacity is indicative of damage to the cornea. Opacity can be evaluated

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[7] The definitions in this glossary are restricted to their uses with respect to the AMCP test methods and testing strategy.

[8] Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003).

subjectively as done in the Draize rabbit eye test, or objectively with an instrument such as an opacitometer.

**Corneal permeability:** Quantitative measurement of damage to the corneal epithelium by a determination of the amount of sodium fluorescein dye that passes through all corneal cell layers.

**Corrosion:** Destruction of tissue at the site of contact with a substance.

**Corrosive:** A substance that causes irreversible tissue damage at the site of contact.

**Endpoint:**[8] The biological process, response, or effect assessed by a test method.

**Essential test method components:**[8] Structural, functional, and procedural elements of a test method that are used to develop the test method protocol. These components include unique characteristics of the test method, critical procedural details, and quality control measures. Adherence to essential test method components is necessary when the acceptability of a proposed test method is being evaluated based on performance standards derived from mechanistically and functionally similar validated test method. [Note: Previously referred to as *minimum procedural standards*]

**False negative:**[8] A substance incorrectly identified as negative by a test method.

**False negative rate:**[8] The proportion of all positive substances falsely identified by a test method as negative (see *two-by-two table*). It is one indicator of test method accuracy.

**False positive:**[8] A substance incorrectly identified as positive by a test method.

**False positive rate:**[8] The proportion of all negative substances that are falsely identified by a test method as positive (see *two-by-two table*). It is one indicator of test method accuracy.

**Globally Harmonized System (GHS):** A classification system presented by the United Nations that provides (a) a harmonized criteria for classifying substances and mixtures according to their health, environmental and physical hazards and (b) harmonized hazard communication elements, including requirements for labeling and safety data sheets.

**Good Laboratory Practice (GLP):**[8] Regulations promulgated by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency, and principles and procedures adopted by the OECD and Japanese authorities, which describe record keeping and quality assurance procedures for laboratory records that will be the basis for data submissions to national regulatory agencies.

**Hazard:**[8] The potential for an adverse health or ecological effect. Hazard potential results only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

**Interlaboratory reproducibility:**[8] A measure of whether different qualified laboratories using the same protocol and test substances can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the prevalidation and validation processes and indicates the extent to which a test method can be transferred successfully among laboratories.

**Intralaboratory repeatability:**[8] The closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period.

**Intralaboratory reproducibility:**[8] The first stage of validation; a determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

***In vitro:*** In glass; Refers to test methods that are carried out in an artificial system (e.g., in a test tube or petri dish) and typically use single-cell organisms, cultured cells, cell-free extracts, or purified cellular components.

***In vitro* irritancy score (IVIS):** An empirically derived formula used in the BCOP test method whereby the mean opacity and mean permeability values for each treatment group are combined into a single *in vitro* score for each treatment group. The *in vitro* irritancy score = mean opacity value + (15 x mean permeability value).

***In vivo:*** In the living organism. Refers to test methods performed in multicellular organisms.

**Iris:** The contractile diaphragm perforated by the pupil and forming the colored portion of the eye.

**Negative predictivity:**[8] The proportion of correct negative responses among substances testing negative by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Negative predictivity is a function of the sensitivity of the test method and the prevalence of negatives among the substances tested.

**Nonirritant:** (a) A substance that produces no changes in the eye following its application to the anterior surface of the eye. (b) Substances that are not classified as GHS Category 1, 2A, or 2B; or EU R41 or R36 ocular irritants.

**Nonsevere irritant:** (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye; the tissue damage is reversible within 21 days of application and the observed adverse effects in the eye are less severe than observed for a severe irritant. (b) Substances that are classified as GHS Category 2A or 2B; EPA Category II, III, or IV; or EU R36 ocular irritants.

**Ocular:** Relating to the eye.

**Ocular corrosive:** A substance that causes irreversible tissue damage in the eye following application to the anterior surface of the eye.

**Ocular irritant:** A substance that produces a reversible change in the eye following application to the anterior surface of the eye.

**Opacitometer:** An instrument used to measure "corneal opacity" by quantitatively evaluating light transmission through the cornea. The instrument has two compartments, each with its own light source and photocell. One compartment is used for the treated cornea, while the other is used to calibrate and zero the instrument. The difference between photocell signals in the two compartments is measured electronically as a change in voltage, and is displayed digitally, generating numerical opacity values with arbitrary units.

**Pannus:** A specific type of corneal inflammation that begins within the conjunctiva, and with time spreads to the cornea. Also referred to as "chronic superficial keratitis."

**Performance:**[8] The accuracy and reliability characteristics of a test method (see *accuracy*, *reliability*).

**pH:** A measure of the acidity or alkalinity of a solution. pH 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

**Positive control:** A substance known to induce a positive response used to demonstrate the sensitivity of the test method and to allow for an assessment of variability in the conduct of the test method over time. For most test methods, the positive-control substance is tested concurrently with the test substance and the vehicle/solvent control. However, for some *in vivo* test methods, periodic studies using a positive-control substance is considered adequate by the OECD.

**Positive predictivity:**[8] The proportion of correct positive responses among substances testing positive by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Positive predictivity is a function of the sensitivity of the test method and the prevalence of positives among the substances tested.

**Prevalence:**[8] The proportion of positives in the population of substances tested (see *two-by-two table*).

**Protocol:**[8] The precise, step-by-step description of a test, including the listing of all necessary reagents, criteria, and procedures for the evaluation of the test data.

**Quality assurance:**[8] A management process by which adherence to laboratory testing standards, requirements, and record keeping procedures is assessed independently by individuals other than those performing the testing.

**Reduction alternative:**[8] A new or modified test method that reduces the number of animals required.

**Reference test method:**[8] The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

**Refinement alternative:**[8] A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal wellbeing.

**Relevance:**[8] The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the *accuracy* or *concordance* of a test method.

**Reliability:**[8] A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and interlaboratory reproducibility and intralaboratory repeatability.

**Replacement alternative:**[8] A new or modified test method that replaces animals with non-animal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

**Reproducibility:**[8] The consistency of individual test results obtained in a single laboratory (*intralaboratory reproducibility*) or in different laboratories (*interlaboratory reproducibility*) using the same protocol and test substances (see intra- and *interlaboratory reproducibility*).

**Sclera:** The tough, fibrous tissue that extends from the cornea to the optic nerve at the back of the eye.

**Secondary bacterial keratitis:** Inflammation of the cornea that occurs secondary to another insult that compromised the integrity of the eye.

**Sensitivity:**[8] The proportion of all positive substances that are classified correctly as positive in a test method. It is a measure of test method accuracy (see *two-by-two table*).

**Severe irritant:** (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye that is not reversible within 21 days of application or causes serious physical decay of vision. (b) Substances that are classified as GHS Category 1, EPA Category I, or EU R41 ocular irritants.

**Solvent control:** An untreated sample containing all components of a test system, including the solvent that is processed with the test substance-treated and other control samples to establish the baseline response for the samples treated with the test substance dissolved in the same solvent. When tested with a concurrent negative control, this sample also demonstrates whether the solvent interacts with the test system.

**Specificity:**[8] The proportion of all negative substances that are classified correctly as negative in a test method. It is a measure of test method accuracy (see *two-by-two table*).

**Test:**[8] The experimental system used; used interchangeably with *test method* and *test method*.

**Test method:**[8] A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a substance or agent to produce a specified biological effect under specified conditions. Used interchangeably with *test* and *test method*. See also *validated test method* and *reference test*.

**Tiered testing:** A testing strategy where all existing information on a test substance is reviewed, in a specified order, prior to *in vivo* testing. If the irritancy potential of a test substance can be assigned, based on the existing information, no additional testing is required. If the irritancy potential of a test substance cannot be assigned, based on the existing information, a step-wise animal testing procedure is performed until an unequivocal classification can be made.

**Toxic keratoconjunctivitis:** Inflammation of the cornea and conjunctiva due to contact with an exogenous agent. Used interchangeably with *contact keratoconjunctivitis*, *irritative keratoconjunctivitis*, and *chemical keratoconjunctivitis*.

**Transferability:**[8] The ability of a test method or procedure to be accurately and reliably performed in different, competent laboratories.

**Two-by-two table:**[8] The two-by-two table can be used for calculating accuracy (concordance) ([a+d]/[a+b+c+d]), negative predictivity (d/[c+d]), positive predictivity (a/[a+b]), prevalence ([a+c]/[a+b+c+d]), sensitivity (a/[a+c]), specificity (d/[b+d]), false positive rate (b/[b+d]), and false negative rate (c/[a+c]).

| | | **New Test Outcome** | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Reference Test Outcome** | Positive | a | c | a + c |
| | Negative | b | d | b + d |
| | **Total** | **a + b** | **c + d** | **a + b + c + d** |

**Validated test method:**[8] An accepted test method for which validation studies have been completed to determine the relevance and reliability of this method for a specific proposed use.

**Validation:**[8] The process by which the reliability and relevance of a procedure are established for a specific purpose.

**Vehicle control:** An untreated sample containing all components of a test system, including the vehicle that is processed with the test substance-treated and other control samples to establish the baseline response for the samples treated with the test substance dissolved in the same vehicle.

**Weight of evidence (process):** The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.